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**Final Technical Report**

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**Cooperative Agreement NCC 2-534**

**Project Title:** Towards a Self-Replicating Chemical System Based on  
Cytidylic and Guanylic Acids

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**Submitted by:** The Regents of the University of California, University of  
California, Santa Cruz (UCSC).

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NASA/Ames Res. Ctr.

Enclosure: Final Technical Report and two reprints

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SELF-REPLICATING CHEMICAL SYSTEM  
BASED ON CYTIDYLIC AND GUANYLIC  
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## Final Technical Report, Cooperative Agreement NCC 2-534

Towards a Self-Replicating Chemical System Based on Cytidylic and Guanylic Acids

Our proposal addressed two areas important for the understanding of template-directed reactions and necessary for the development of a self-replicating chemical system based on nucleotides. The objectives of the proposed research were: (1) To elucidate the mechanistic aspects of template-directed chemistry and to identify the conditions, environmental and other, that favor "organized chemistry" and stereoselective polymerization of nucleotides; (2) To initiate a search for catalysts to improve the efficiency of these reactions. Enhanced efficiency is expected to facilitate the road towards a self-replicating chemical system based on all four nucleic acid bases.

Our efforts since January 1988 in the above areas have been very fruitful. The work resulted in two papers. Furthermore, some of our experiments lead to the identification of new and exciting areas of research. Below we present the summaries of the two papers that are enclosed with this report.

1. Anastassia Kanavarioti, Claude F. Bernasconi, Donald L. Doodokyan and Diann J. Alberas, "Magnesium Ion Catalyzed P-N Bond Hydrolysis in Imidazolidine-Activated Nucleotides. Relevance to Template-Directed Synthesis of Polynucleotides." *J. Am.Chem. Soc.* **1989**, *111*, 7247.

**Summary:** Magnesium, an ion necessary in enzymatic as well as in nonenzymatic template-directed polynucleotide-synthesizing reactions, has been found to catalyze the hydroxide ion attack on the P-N bond of selected 5'-monophosphate imidazolidine derivatives of nucleotides, such as guanosine 5'-monophosphate 2-methylimidazolidine (2-MeImpG), guanosine 5'-monophosphate imidazolidine (ImpG), and adenosine 5'-monophosphate 2-methylimidazolidine (2-MeImpA). Calcium ion behaves similarly, but quantitatively the effects are smaller. Pseudo-first-order rate constants of 2-MeImpG and ImpG hydrolysis as a function of  $Mg^{2+}$  concentration have been obtained in the range  $6 \leq pH \leq 10$  at  $37^\circ C$ .  $Mg^{2+}$  catalysis is particularly effective around pH 10 where a 0.02 M concentration leads to 15-fold acceleration and a 0.2 M concentration to a 115-fold acceleration of the rate. At other pH values  $Mg^{2+}$  catalysis is less dramatic, mainly because the noncatalyzed reaction is faster.  $Mg^{2+}$  catalysis is attributed to the reaction of the zwitterionic form of the substrate ( $SH^\pm$ , imidazolidine moiety protonated) with  $OH^-$  rather than the reaction of the anionic

form ( $S^-$ , imidazolid moiety deprotonated) with water. This conclusion is based on a study of the N-methylated substrates N-MeImpG and 1,2-diMeImpG, respectively, which were generated in situ by the equilibrium reaction of ImpG with N-methylimidazole and 2-MeImpG with 1,2-dimethylimidazole, respectively. In contrast, in the absence of  $Mg^{2+}$  the reaction of  $S^-$  with water competes with the reaction of  $SH^\pm$  with  $OH^-$ . The present study bears on the mechanism of the  $Mg^{2+}$ -catalyzed template-directed synthesis of oligo- and polynucleotides derived from 2-MeImpG and on the competition between oligonucleotide synthesis and hydrolysis of 2-MeImpG.

2. Anastassia Kanavarioti and Claude F. Bernasconi, "Computer Simulation in Template-Directed Oligonucleotide Synthesis" *J. Mol. Evol.* **1990**, *31*, 470.

**Summary:** A computer simulation (KINSIM) modeling up to 33 competing reactions was used in order to investigate the product distribution in a template-directed oligonucleotide synthesis as a function of time and concentration of the reactants. The study is focused on the poly(C)-directed elongation reaction of an oligoguanylate (a 7-mer is chosen) with guanosine 5'-monophosphate-2-methylimidazolid (2-MeImpG), the activated monomer. It is known that the elongation of oligoguanylates to form oligomeric products such as 8-mer, 9-mer, 10-mer etc. is in competition with (i) the dimerization and further oligomerization reaction of 2-MeImpG that leads to the formation of dimers and short oligomers, and (ii) the hydrolysis of 2-MeImpG that forms inactive guanosine 5'-monophosphate, 5'-GMP. Experimentally determined rate constants of the above three processes at 37°C and pH 7.95 were used in the simulation; the initial concentrations of 2-MeImpG,  $[M]_0$ , and of the oligoguanylate primer,  $[7\text{-mer}]_0$ , were varied and KINSIM calculated the distribution of products as a function of time until equilibration was reached, i.e., when all the activated monomer has been consumed. In order to sort out how strongly the elongation reaction may be affected by the competing hydrolysis and dimerization, we also simulated the idealized situation in which these competing reactions do not occur. Simulation of the idealized system suggests that (a) the fraction of  $[7\text{-mer}]_0$  that has reacted as well as the product distribution after equilibration do not depend on the absolute concentrations of the reactants, but only on their ratio,  $[M]_0/[7\text{-mer}]_0$ ; (b) the rate of elongation is proportional to  $[7\text{-mer}]_0$  and not to  $[M]_0$ ; (c) as the  $[M]_0/[7\text{-mer}]_0$  ratio increases longer oligomers are formed. The results of the computer simulation with the experimental system, i.e. elongation in the presence of both hydrolysis and dimerization, are similar to the ones obtained with the idealized system as long as dimerization and hydrolysis are not responsible for consuming a substantial fraction of 2-MeImpG.